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COMPARISON OF CROSS-LINKED HEMOGLOBIN SOLUTION TO LACTATED RINGERS AND 5% ALBUMIN IN RESUSCITATION OF A RAT MODEL OF *ESCHERICHIA COLI* SEPTIC SHOCK

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TECHNICAL REVIEW AND APPROVAL

NMRI 93-81

The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

ROBERT G. WALTER
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13. ABSTRACT *(Maximum 200 words)*

Intramolecularly cross-linked hemoglobin (lysine 99 alpha 1-lysine 99 alpha 2 cross-linkage) has been reported to have oxygen transport properties similar to those of whole blood. Partial and complete transfusions in swine have demonstrated that cross-linked hemoglobin solutions transport oxygen and allow normal cardiac and renal function in the virtual absence of red blood cells. Recently, a study of this cross-linked hemoglobin solution in a rat model of hemorrhagic shock showed an improved hemodynamic response to cross-linked hemoglobin solution over that provided by lactated Ringer's (R/L) solution. The hemodynamic response was similar to that of autologous whole blood infusion. In the present study we have attempted to utilize a cross-linked hemoglobin solution in resuscitation of a rat septic shock model. Sprague-Dawley rats were anesthetized with ketamine and injected i.p. with E. coli. Two hours after E. coli challenge the animals were resuscitated with either 7% cross-linked hemoglobin, 5% albumin or R/L at 10 cc/kg or 40 cc/kg. Hemodynamic parameters were monitored for up to five hours on the anesthetized animals. There were no significant differences between any treatment groups for heart rate, mean arterial pressure (MAP), cardiac output, or regional blood flow to tissues. In conclusion, the true benefits of stroma-free hemoglobin (SFH) resuscitation could not be properly assessed in this pilot study because of the role of ketamine anesthesia and fluid maintenance given to the animals prior to the administration of the SFH, human albumin (HA), and R/L resuscitative solutions. During the course of this experiment additional in vitro studies were done that showed that SFH potentiated the growth of pathogenic bacteria. Therefore, an awake model would be better suited to determine the advantages or disadvantages of SFH used in sepsis.

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This study was approved by the Animal Care and Use Committee, Naval Medical Research Institute, Septic Shock Research Department and the experiments reported herein were conducted according to the principles set forth in the " Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animals Resources, National Research Council, DHHS, Pub.No.(NIH) 86-23 (1985)

The authors express their sincere appreciation to HM2 Brian Hendricks, HM2 John Kelly, HM2 Anna Wakefield and Mrs. Alesia Holly, for their expert technical assistance in the completion of this work. As well, the excellent editorial assistance of Mr. Edd Olds is appreciated.

The authors also wish to express their sincere appreciation to Baxter-Travenol for their donation of the hemoglobin solution that was used in the experiments.

INTRODUCTION

Septic patients may present with hypoperfusion, as evidenced by low blood pressure, cool extremities, delayed capillary refill time, and altered mentation. When these patients are evaluated by invasive hemodynamic monitoring, they exhibit a hypodynamic profile, with low cardiac index and high systemic vascular resistance (1). Patients manifesting this hemodynamic low flow state will not survive unless treatment is rapidly instituted with large volumes of intravenous fluids to help reverse the hypodynamic or hypotensive condition. This intervention is believed to lead to a hyperdynamic state characterized by high cardiac index and low systemic vascular resistance often associated with sepsis (1-3). The debate over the optimal type of resuscitative fluids to use in the treatment of shock has a long history and remains unresolved. Recently, hemoglobin solutions that are cell-membrane-free (classically termed "stroma-free hemoglobin", abbreviated hereafter as SFH), have shown great promise as resuscitative fluids. These solutions have high oxygen carrying capacity, elevated colloid oncotic pressure, low viscosity, long shelf-life, and can be readily purified of bacterial and viral contamination (2).

Use of hemoglobin solutions as a blood substitute has been thwarted by the serious problem of high oxygen affinity in solutions low in the organic ligand, 2,3-diphosphoglycerate (2,3 DPG). The 2,3 DPG is often lost during preparative procedure, and the resultant solutions have an oxygen affinity that is too

high for clinically useful oxygen off-loading. Further, to be clinically functional as a blood substitute, SFH must be retained in the plasma and be able to bind and release oxygen at appropriate oxygen tensions (3,4). Various modifications of SFH have been prepared in order to overcome these deficiencies.

Intramolecularly cross-linked hemoglobin (lysine 99 alpha 1-lysine 99 alpha 2 cross-linkage) has been reported to have oxygen transport properties similar to those of whole blood (5). The cross-linking of alpha subunits provides the hemoglobin with a favorable (right-shifted) oxygen dissociation curve (6-8) and stabilizes the dimer, thereby prolonging intravascular retention (9,10). Partial and complete transfusions in swine have demonstrated that cross-linked hemoglobin solutions transport oxygen and allow normal cardiac and renal function in the virtual absence of red blood cells (11). Recently, a study of this cross-linked hemoglobin solution in a rat model of hemorrhagic shock showed an improved hemodynamic response to cross-linked hemoglobin solution over that provided by lactated Ringer's solution. The hemodynamic response was similar to that of autologous whole blood infusion (12). While hemorrhagic shock is different from septic shock, in the present study we have attempted to utilize a cross linked hemoglobin solution in resuscitation of a rat septic shock model.

MATERIALS AND METHODS

Materials:

The SFH solution and lactated Ringer's (R/L) used in these experiments were obtained from Baxter-Travenol Company. The hemoglobin was prepared as 7% hemoglobin diluted in lactated Ringer's solution in strictly sterile laboratory conditions. The hemoglobin solution was consistently found to be without lipid or endotoxin contamination (Baxter-Travenol). Human albumin (HA) used was obtained as a five-percent solution, from Armour Pharmaceutical Company (Kankakee, IL). Radiolabelled microspheres were purchased from New England Nuclear Products Company (Boston, MA).

Surgical Preparation:

Male Sprague-Dawley rats weighing 300-450g were used in this study and subjected to Escherichia coli strain B7 (086a:K61, ATCC #33985) peritonitis-induced septic shock. Ketamine was used to induce and maintain anesthesia for the surgical and experimental procedure. An initial intra-peritoneal injection of ketamine at 150 mg/kg body weight induced a surgical plane of anesthesia. A ventral incision at the base of the rat tail allowed access to the tail artery and a polyethylene cannula (PE-50; interior diameter 0.58 mm, exterior diameter 0.965 mm; Clay Adams Division, Becton Dickson and Co., Parsippany, NJ) was advanced into the artery for about 1 cm. Through a ventral cervical midline incision, the right external jugular vein was catheterized in similar fashion. Another catheter was advanced

into the left ventricle via the right carotid artery. Its placement was confirmed by characteristic standard pressure tracings. All incisions were closed with staples and a maintenance intravenous infusion of 5% dextrose in normal saline was started at 4 ml/kg/h via the external jugular vein. To this maintenance fluid supplemental ketamine was added to provide a continuous infusion of 40 mg/kg/h throughout the experiment. The animals were then placed in a prone position.

Protocol:

After instrumentation, a one hour post operative recovery period baseline of heart rate, arterial blood pressure and regional blood flows were obtained. Rats then received an intraperitoneal injection of E. coli (2 X 10^{10} cfu/kg, approximately an LD 80) (see Figure 1). All hemodynamic and blood flow parameters were measured at 2, 2.5, 3, and 5 h after the onset of sepsis. (see Figure 4 for a summary of the interventions).

Hemodynamics were obtained using pressure transducers (Spectramed Inc. model P23XL and P10EZ transducers, Oxnard, CA), and measurements were processed through a commercially available data acquisition software program ("CODAS" by Dataq Instruments, Inc., Akron, OH).

Blood flow measurements:

The use of radiolabelled microspheres allows determination of cardiac output, organ blood flow and regional distribution of cardiac output (13,14). Simultaneous measurement of oxygen

content (CaO2) allowed oxygen delivery to individual organs to be estimated.

The microspheres used were approximately 15 microns in size and are made of a latex polymer. They were "tagged" with one of five isotopes: Cerium 141 (Ce 141), Strontium 85 (Sr 85), Scandium 46 (Sc 46), Tin 113 (Sn 113), Chromium 51 (Cr 51). Microsphere suspensions were sonicated and vortexed prior to injection into the left ventricle. At the specified time each of the five radiolabelled microsphere sets was injected over 15 seconds and the catheter was flushed. At the beginning of the microsphere injection blood was withdrawn from the tail artery at a constant rate of 0.45 ml/min(by a Harvard Apparatus infusion-withdrawal pump) for 90 seconds to serve as a reference for determination of cardiac outputs and regional blood flow calculations. After obtaining this sample, 0.3 cc of blood was obtained for determination of oxygen content using a cooximeter (Instruments Laboratory). At the end of the experiment, the animal was euthanized with intravenous ketamine, and the following organs were harvested: heart, lungs, brain, kidneys, adrenals, spleen, pancreas, stomach, small bowel, colon, and testes.

Each of these samples was weighed and placed in vials for measurement of radioactivity by a gamma scintillation counter. By determining cardiac output and oxygen content at a given time, and the amount of microspheres deposited in each tissue, individual organ blood flow and oxygen delivery can be estimated.

By using different radiolabelled microspheres at different times, the time course of regional blood flow and oxygen delivery was determined in the same animal. Calculations of cardiac output and regional blood flow were performed using a software program written for the analysis of multiple isotopes (Law WR, Naval Medical Research Institute, Bethesda, MD.).

Fluid Resuscitation:

According to random group designation at two hours post E. coli injection, each animal had blood samples withdrawn and then received either **10** or **40** ml/kg of resuscitative fluid intravenously over 20 minutes. The resuscitative fluids were either 1) 5% albumin (HA), 2) 7% cross-linked hemoglobin solution (SFH), or 3) Lactated Ringer's solution (R/L). HA solution is commonly used as a resuscitative agent in human septic shock and has similar colloidal osmotic pressure as SFH, therefore it was chosen for comparison with SFH. Hemodynamic and blood flow measurements were taken at 2.5 and 5 hours after septic challenge. The surviving animals were euthanized with intravenous ketamine after the 5 h measurements were made. Organs of interest were then harvested and evaluated for gamma radioactivity.

Controls:

One control group received only maintenance fluids and ketamine anesthesia. A second control group received intraperitoneal (ip) E. coli, maintenance fluids and ketamine anesthesia. The number of animals in each group is shown in

Table 1.

In Vitro Studies:

Bacterial growth in SFH and HA solutions. Various concentrations of SFH or HA solutions were made in brain heart infusion (BHI, Difco, Detroit, MI) broth and inoculated with E. coli strain B7. The optical densities (O.D.) of the growing cultures were measured with time and compared with cultures in BHI alone (Figures 7 and 8). SFH solutions containing an iron chelator, desferoxamine mesylate (Desferal, Ciba-Geigy, Summit NJ), were also examined (Figure 9).

RESULTS AND DISCUSSION

*Preliminary Data:

Figure 1 shows the percent mortality over 24 hours for rats after challenged with increasing bacterial counts. It should be noted that this preliminary mortality and morbidity study was performed on awake, fully conscious rats after a 24 hour recovery from their surgical instrumentation. The group which received intra-peritoneal E. coli at 2 x 10^{10} cfu/kg showed nearly 80% mortality within 24 h, (Figure 1), and manifested early tachycardia and hypotension (Figures 2 and 3).

The data from the above preliminary study on awake rats was used to determine the inoculum of E. coli that would most likely cause hemodynamic alterations during the first few hours after intra-peritoneal injection of the organism and yet allow animals to survive the experiment at least 6 h or more after induction of peritonitis. Similar control data in anesthetized animals were not undertaken. The "confounding variables" that we may have encountered by making predictions in this fashion are discussed subsequently. After a consultation with critical care experts, the principal investigators of this project decided to use ketamine as the sole anesthetic for induction and maintenance of anesthesia throughout the course of the experiments since it was concluded that this drug was least likely to influence hemodynamics in this model.

Observations:

There were no big differences in heart rate aMAP between any of the treatment groups (Figures 5 and 6). Rates were elevated due to anesthetic and remain high throughout. Further, there were no significant differences in CO and regional blood flow between any of the treatment groups, thus only representation group values any presentation, Tables 2 - 5. CO generally exhibits a steady decline over the time course in all groups tested. There were no consistent trends in regional blood flow. It must be noted that the anesthetized animals received ketamine in a continuous infusion throughout the experiment.

While ketamine is less likely to cause acute cardiac depression than any other inhaled or intravenous anesthetics, it may cause an initial increase in myocardial performance by stimulating endogenous catecholamine release. This might inhibit, or cause a delay in the hypotension seen in the awake model following E. coli injection.

***The preliminary data obtained in this anesthetized rat septic shock model will be used for an experimental awake rat septic model planned by Dr. William R. Law, University of Illinois, School of Medicine, Chicago, Illinois.**

The anesthetized animals also received maintenance fluids at a rate of 4 ml/kg/h in order to offset the lack oral intake that was allowed in the awake model must also be a considered as an additional contributing component for fluid resuscitation and combined with the myocardial effects of ketamine, may have inhibited or delayed the hemodynamic abnormalities normally seen following injection of E. coli into the peritoneum of awake, non-anesthetized animals. Another confounding variable in comparison of the awake versus the anesthetized models, involves a 24 h post surgical period for the awake model, as opposed to only a one hour period for the anesthetized model. Inflammatory mediators, induced by the surgical procedure may have been in the midst of the inflammatory cascade as sepsis was induced in the anesthetized model. The awake model was allowed approximately 23 more hours of recovery from the surgery than the anesthetized model was before peritonitis/sepsis was induced. To summarize the above, there was an inhibition or time delay in the hemodynamic response following peritonitis-induced sepsis in the ketamine anesthetized animals possibly due to the administration of maintenance intravenous fluids, ketamine-induced endogenous catecholamine response, and/or the lack of a post surgical recovery period. There were no differences in hemodyn parameters between any of the treatment groups.

There were several deaths during the course of the experiment. Although this was not set up as a lethality study, groups had an apparent increase in mortality (Figures 10 and 11).

In Figures 5 and 6 it will be noted that some of the error bars are missing in some groups towards the end of the experiment. This is because there was often only one animal alive at the end of the study. By studying the terminal hemodynamics and observing the respiratory effort of animals that died before completion of the experiment, and correlating these events with the increased need for ketamine to maintain analgesia, a likely cause was that the animals developed ketamine toxicity.

Even though a 150 mg/kg of ketamine intraperitoneally was sufficient for induction, and 40 mg /kg/h intravenous bolus of ketamine was initially sufficient for maintenance of a surgical plane of anesthesia, the animals required multiple repeated boluses of ketamine in order to maintain a surgical plane of anesthesia (assessed by interdigital pain response). At the end of the experiment, the animals required more frequent boluses of ketamine to maintain analgesia, and yet would exhibit fairly profound hemodynamic and respiratory suppression when the bolus of ketamine was given. A mechanism by which this could occur involves a rapidly developing tolerance to ketamine, in which the therapeutic index narrows. In this scenario, the amount needed for a therapeutic effect progressively approaches toxic levels. Thus the animal eventually experiences toxicity at doses needed for therapeutic benefits. Apnea, bradycardia and hypotension are terminal side effects of ketamine, and were observed in many of the animals who expired before completion of the experiment.

This phenomenon occurred in some animals of all groups, and Figure 10 shows graphically the number of deaths in each resuscitation group at similar times.

Another mechanism in which ketamine might cause toxicity involves a depletion of endogenous catecholamines as a result of the chronic adrenal stimulation. The catecholamine depletion may interact synergistically with the catecholamine depletion caused over time by sepsis.

These confounding variables tend to diminish any significant conclusions from the results obtained in our fluid resuscitation experiments.

The deaths occurring during or immediately following the fluid resuscitation prompted additional inquiry into the potentiating effects of SFH and HA upon E. coli itself. In vitro, E.coli strain B7 was incubated with varying concentrations of SFH or HA solutions.

The results of this study revealed that E. coli growth was directly correlated with increased concentrations of SFH but not with increased concentration of HA (Figures 7 and 8). In SFH, the onset of log phase growth occurred earlier and resulted in a higher yield. An earlier study has shown that iron in hemoglobin could enhance E. coli virulence in guinea-pigs and that the lethal dose of E. coli decreased by 100,000-fold for guinea-pigs treated with ferric ammonium citrate, an iron source (15). Since our final concentration of 1.4% SFH solution contains approximately 0.8 mM heme-bound iron, we added an iron-chelator

to the SFH containing growth media. Both 5 mM and 10 mM desferoxamine mesylate - labeled "D", caused a dose related inhibition of E. coli growth in SFH (Figure 9). Desferoxamine delayed the onset of log phase growth and reduced total yield in comparison with that achieved in SFH alone. Possibly, iron or hemoglobin bound by desferoxamine in SFH solution may be unavailable or less available to the E. coli.

Since our growth enhancement/inhibition studies were performed in-vitro, their clinical relevance is unknown. The enhanced growth of E. coli when stroma-free hemoglobin is added may indicate a need for caution with the use of this resuscitation fluid in patients who are bacteremic.

In conclusion, the true benefits of SFH resuscitation could not be properly assessed in this pilot study because of the role of ketamine anesthesia and fluid maintenance given to the animals prior to the administration of the SFH, HA and R/L resuscitative solutions. Therefore the awake model, as developed by Dr. Law, would be better suited to determine the advantages or disadvantages of SFH use in sepsis. During the course of this experiment additional in vitro studies were done that showed that SFH potentiated the growth of pathogenic bacteria.

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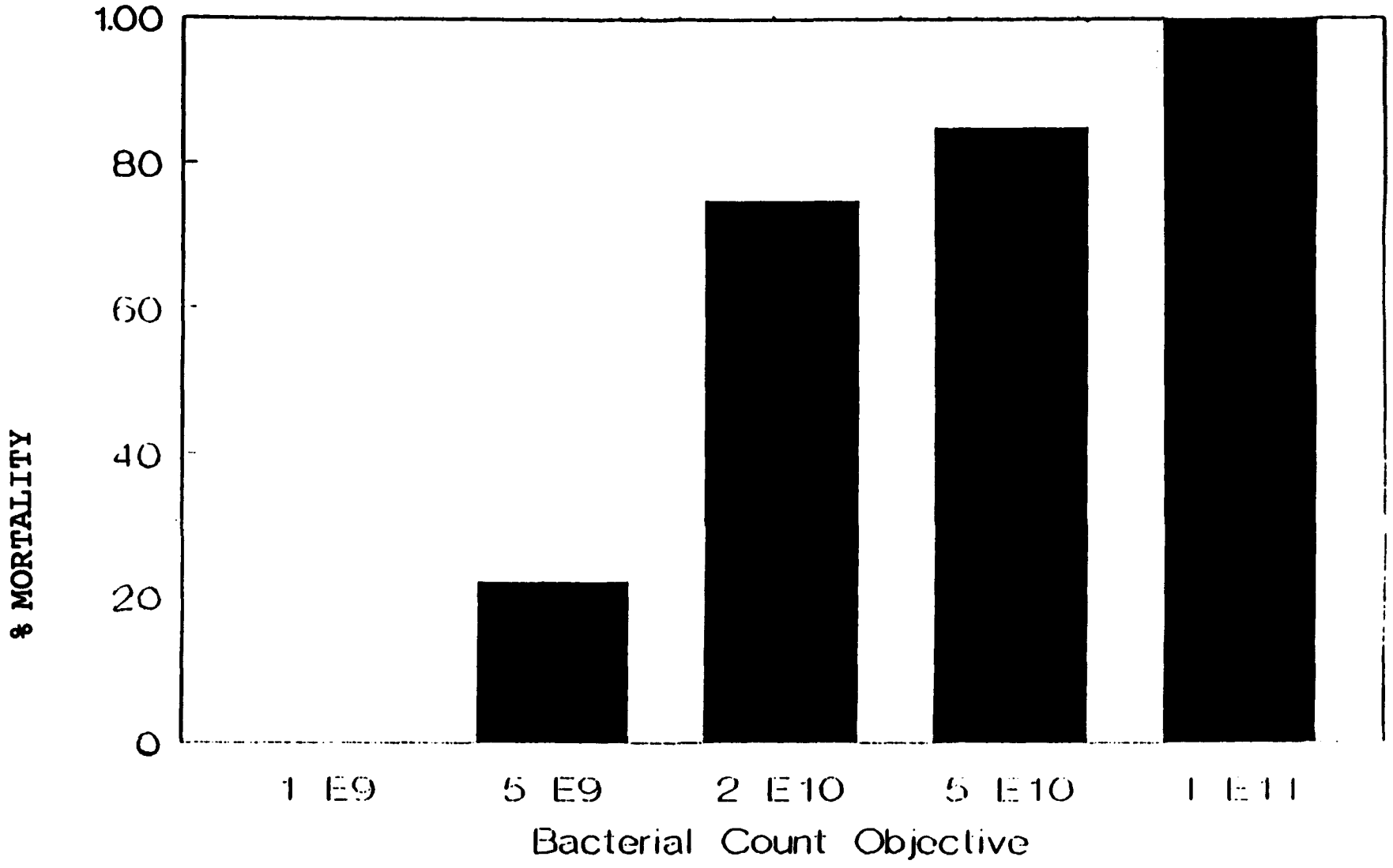

Figure 1. Rat Sepsis Model/Initial Mortality Study **Percent mortality over the 24 hour period following intraperitoneal injection of E. coli. Bacterial levels are expressed as projected E. coli colony forming units(CFU); Six to nine rats were used in each group; no volume resuscitation was provided; A projected CFU of 2 X 10^{10} provided approximately 80% lethality over a 24 hour period, and was used in subsequent experiments.**

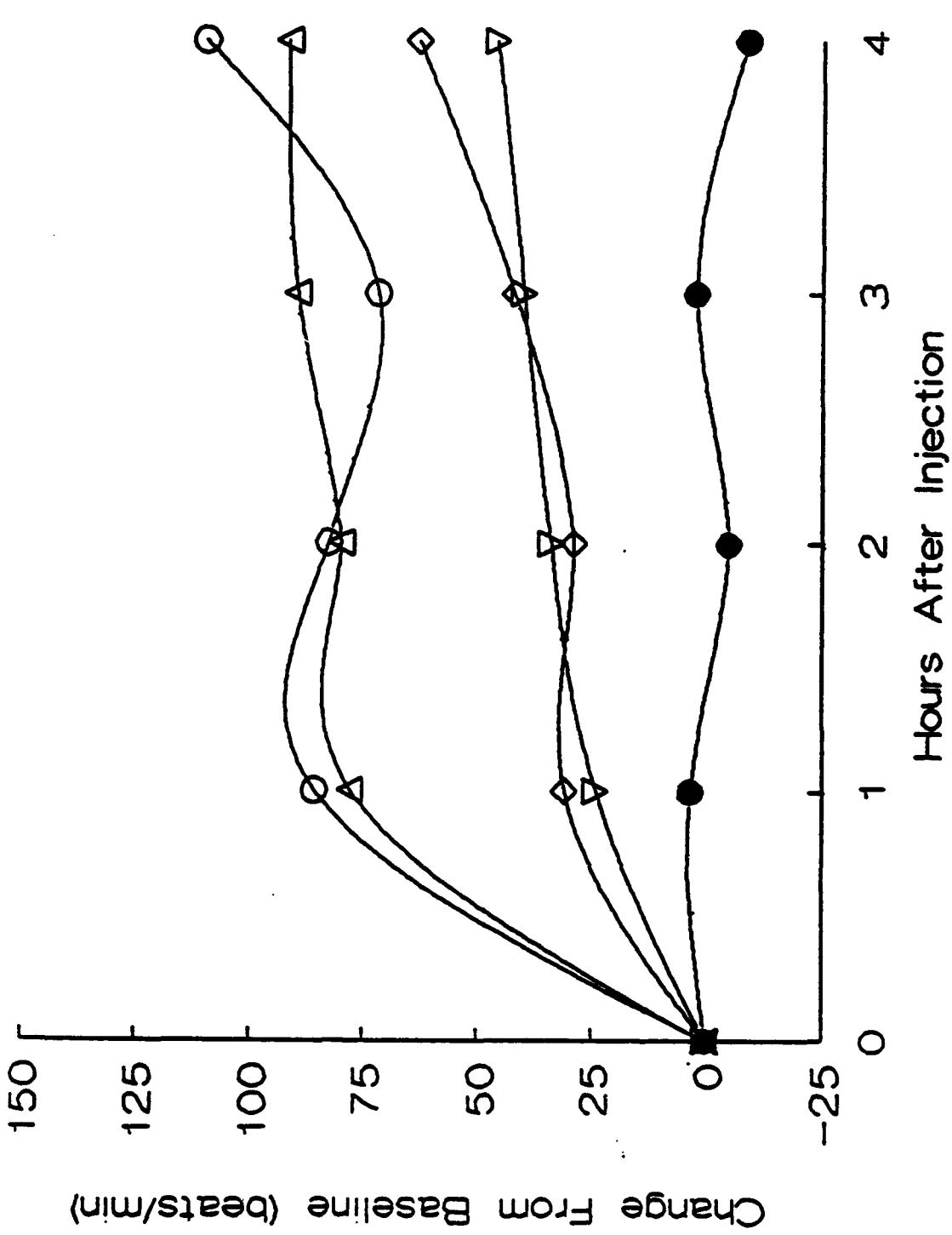

Figure 2. Rat Sepsis Model: Heart Rate: The heart rate percent change from baseline after E. coli intraperitoneal injection. • Saline, ▲ 2E10 CFU, ○ 5E10 CFU, ▼ 1E9 CFU, ◇ 5E9 CFU.

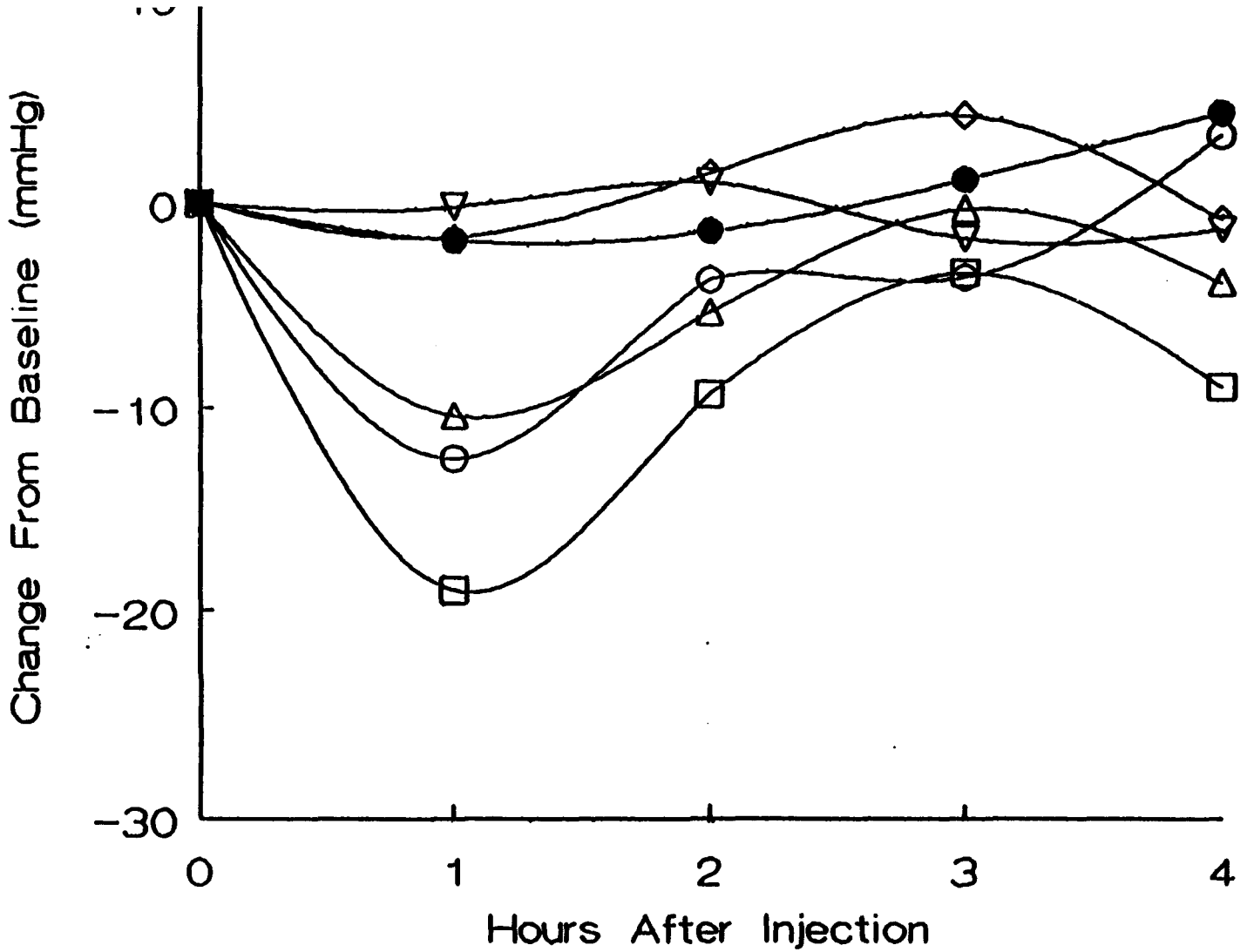

Figure 3. Rat Sepsis Model: Mean Arterial Pressure: The mean arterial blood pressure percent change from baseline after E. coli intraperitoneal injection. • Saline, ▲ 2 E10 CFU, ○ 5 E10 CFU, □ 1E11 CFU, ▼ 1E9 CFU, ◊ 5E9 CFU.

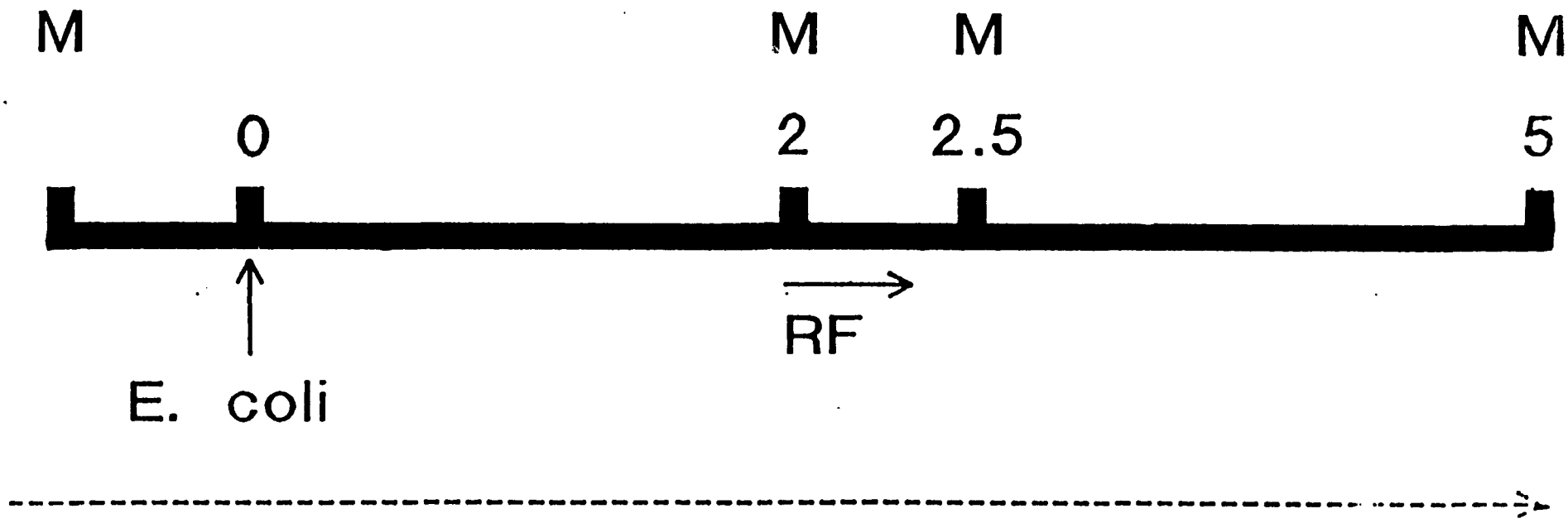

Figure 4. Experimental Protocol "M" indicates the time at which the radiolabelled microspheres were injected to determine regional blood flow, as well as the time that hemodynamics were measured. The numbers on the top line indicate the number of hours after E. Coli was injected into the peritoneum. "RF" indicates resuscitation fluid, which was infused between the 2 and 2.5 hour timepoints. Maintenance fluid, (5% dextrose in 0.45% normal saline), was infused at 4 ml/kg/h throughout the protocol.

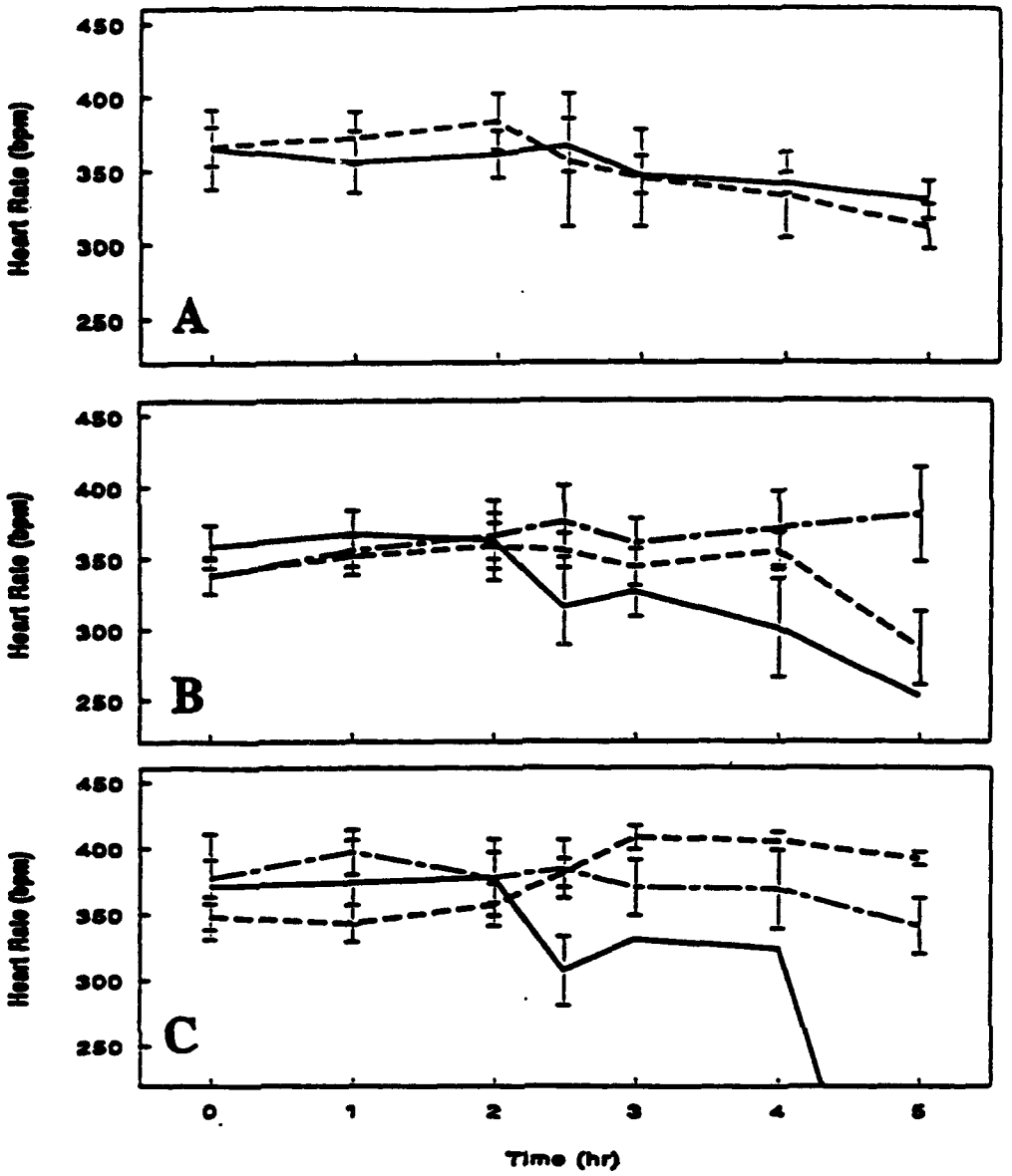

Figure 5. Heart Rate After E. coli or Saline-control Injection The heart rate is expressed in beats per min.

A. No treatment There were no statistically significant differences between the septic (---) and non-septic (—) control groups. The septic group received an intraperitoneal injection of 2 E10 CFU of E. Coli at time zero, while the non-septic control group received an intraperitoneal injection of normal saline at the comparable time.

B. Effect of 10 cc/kg treatment There was no statistically significant difference between the heart rate of septic groups receiving 10 cc/kg intravenous boluses of either 7% hemoglobin (—), Ringer's lactate (·-·) or 5% albumin solution (---), after E. coli injection at time zero. There were insufficient data to make statistical conclusions regarding the 7% hemoglobin solution group at hours 4 and 5.

C. Effect of 40 cc/kg There were no statistically significant difference between the heart rate of 3 septic groups receiving 40 cc/kg intravenous boluses of either 7% hemoglobin (—), Ringer's lactate (·-·) or 5% albumin solution (---), after E. coli injection at time zero. There were insufficient data to make statistical conclusions regarding the 7% hemoglobin solution group at hours 3, 4 and 5.

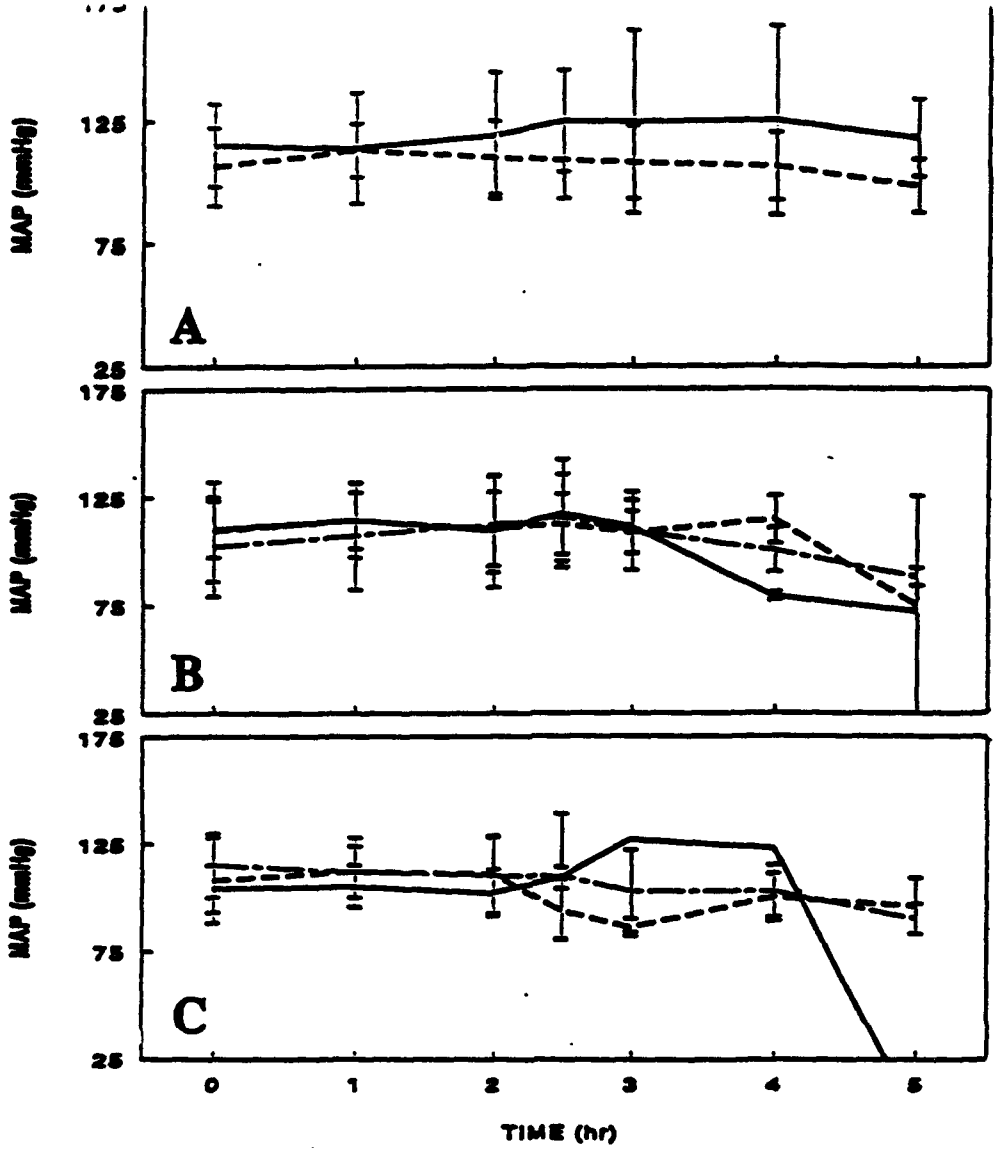

Figure 6. Mean Arterial Pressure After E. coli injection After Saline or E. coli injection The mean arterial pressure expressed as mmHg.

A. No treatment There were no statistically significant difference between the mean arterial pressure of septic (---) and non-septic (—) control groups. The septic group received an intraperitoneal injection of 2 E10 CFU of E. coli at time zero, while the non-septic control group received an intraperitoneal injection of normal saline at the comparable time.

B. Effect of 10 cc/kg treatment There were no statistically significant difference between the mean arterial pressure of 3 septic groups of animals receiving 10 cc/kg intravenous boluses of either 7% hemoglobin (—), Ringer's lactate (·-·) or 5% albumin solution (---), after E. coli injection at time zero. There were insufficient data to make statistical conclusions regarding the 7% hemoglobin solution group at hours 2.5, 4 and 5.

C. Effect of 10 cc/kg treatment There were no statistically significant difference between the mean arterial pressure of 3 septic groups receiving 40 cc/kg intravenous boluses of either 7% hemoglobin (—), Ringer's lactate (·-·) or 5% albumin solution (---), after E. coli injection at time zero. There were insufficient data to make statistical conclusions regarding the 7% hemoglobin solution group at hours 2.5, 3, and 4.

FIGURE 7

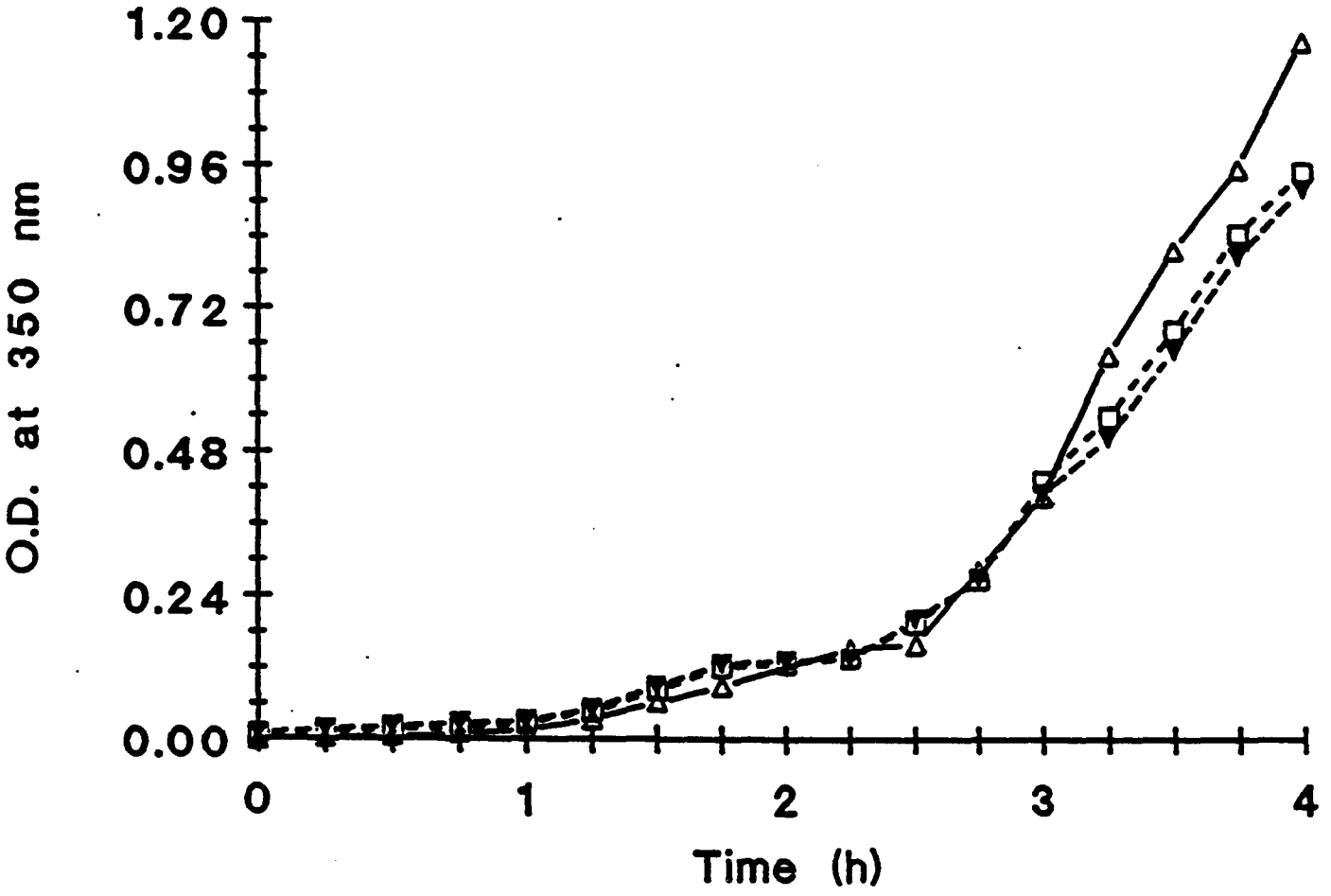

FIG. 7. Effect of human albumin concentration in EHI upon E. coli B7 growth for 4h at 37°C, using 0.0% (△), 1.25% (□), and 1.67% (▼).

FIGURE 8

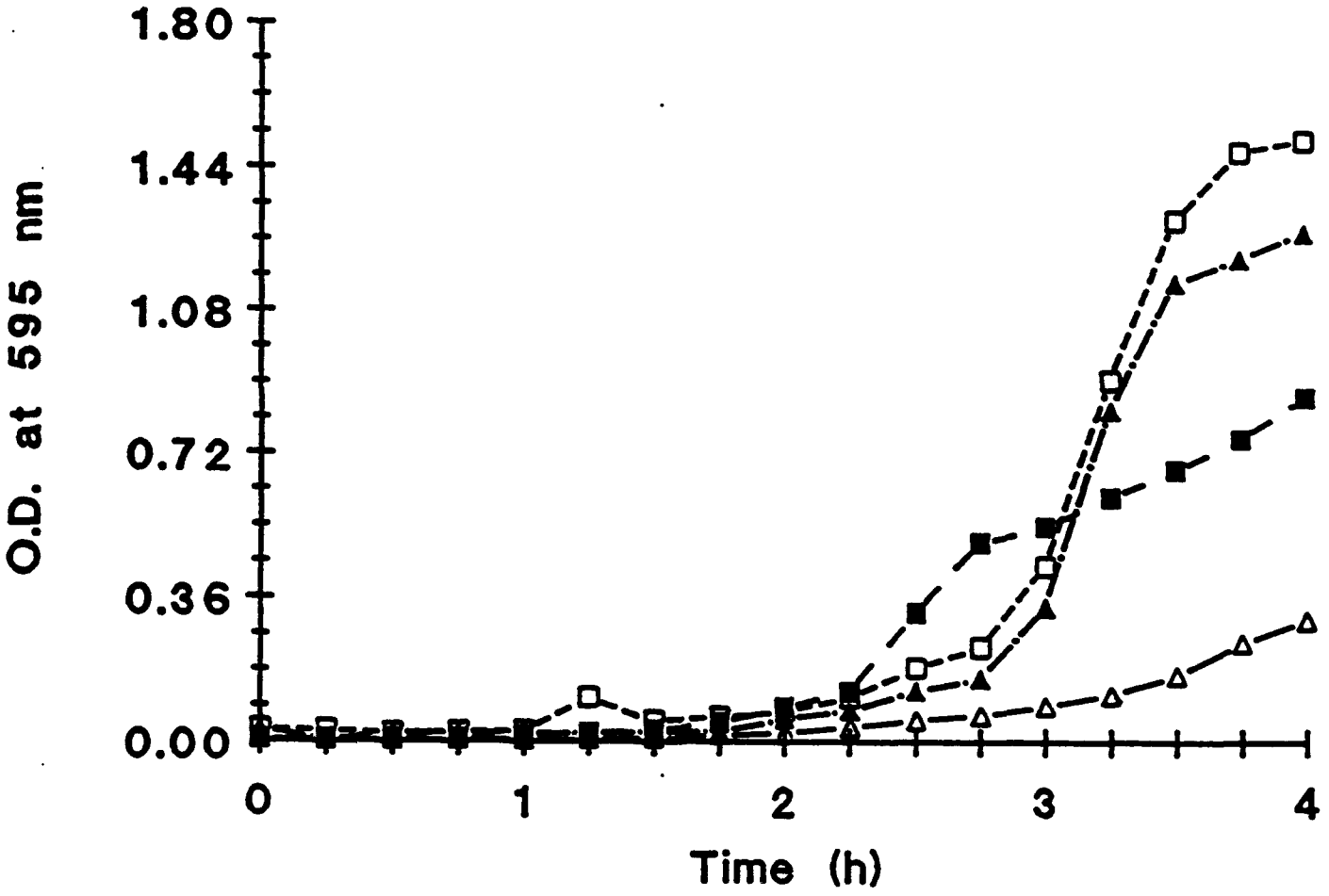

FIG. 8. Effect of SFH concentration in BHI upon E. coli B7 growth for 4h at 37°C, using 0.0% (△), 0.35% (■), 0.70% (▲), and 1.40% (□).

FIGURE 9

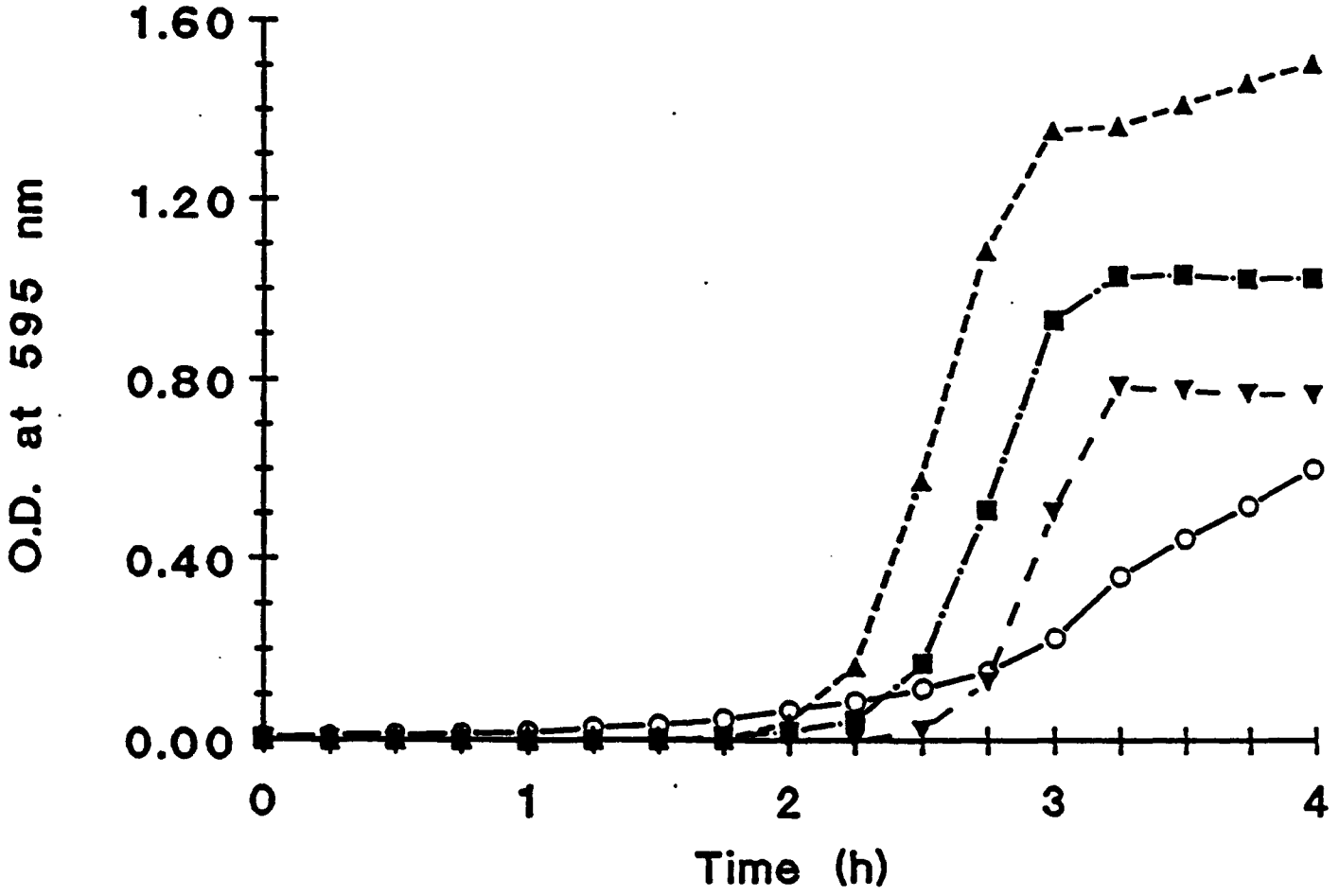

FIG. 9. Effect of Deferoxamine (Desferal) concentration upon E. coli B7 growth for 4h at 37°C in 1.4% SFH solution, using 0mM (▲), 5mM (■), and 10mM (▼), and 0mM in BHI (o).

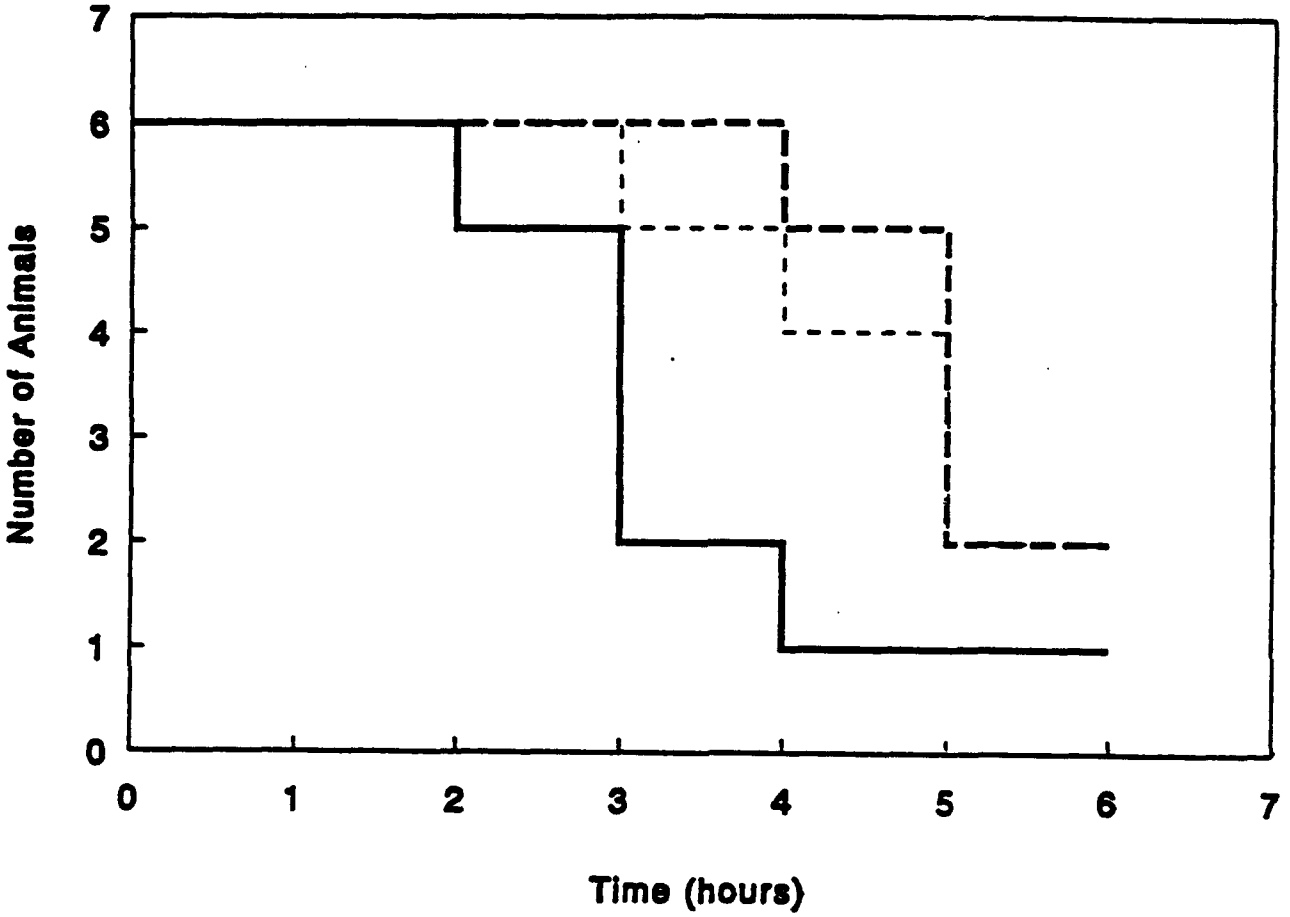

Figure 10. Death Rate The Death Rates of 3 septic groups receiving 10 cc/kg intravenous boluses of either 7% hemoglobin (—), Ringer's lactate (·-·) or 5% albumin solution (---), after E. coli injection at time zero. There were insufficient numbers of animals in each group to make statistical conclusions regarding death rates between groups.

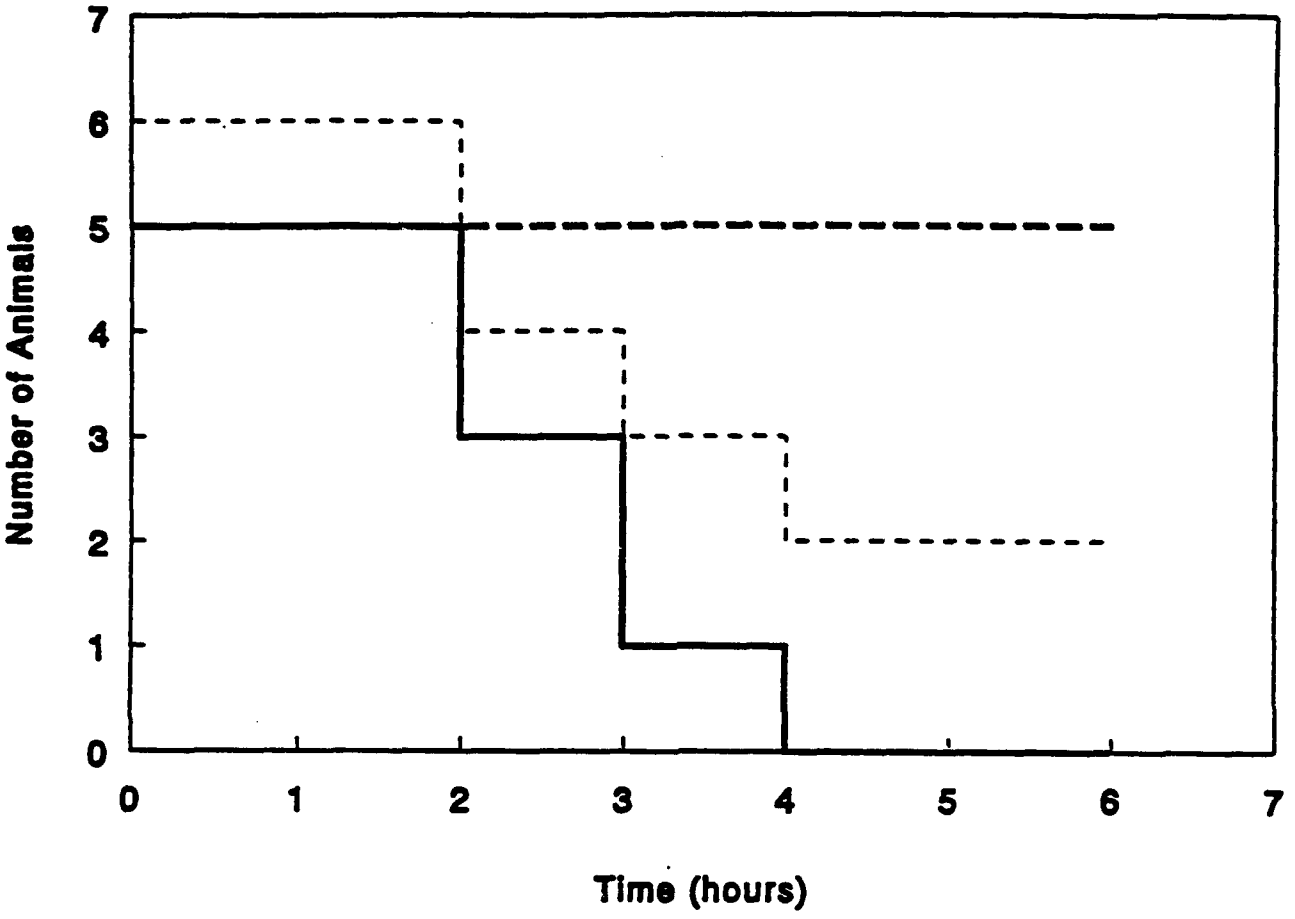

Figure 11. Death Rate Death rates of 3 septic groups receiving 40 cc/kg intravenous boluses of either 7% hemoglobin (—), Ringer's lactate (·-·) or 5% albumin solution (---), after E. coli injection at time zero. There were insufficient numbers of animals in each group to make statistical conclusions regarding death rates between groups.

A. No sepsis / No fluid / No microspheres (NNN)

6 rats

B. No sepsis / No fluid / With microspheres (NNW)

7 rats

C. Sepsis / No fluid / With microspheres (SNW)

7 rats

D. Sepsis / 5% Albumin / With microspheres (SAW)

1. (SAW1 = 40 ml albumin solution per kg rat) 6 rats
2. (SAW2 = 10 ml albumin solution per kg rat) 6 rats

E. Sepsis / 7% Hemoglobin solution / With microspheres (SHW)

1. (SHW1 = 40 ml hemoglobin solution per kg rat) 5 rats
2. (SHW2 = 10 ml hemoglobin solution per kg rat) 7 rats

F. Sepsis / Lactated Ringer's / With microspheres (SLW)

1. (SLW1 = 40 ml lactated Ringers solution per kg rat) 5 rats
2. (SLW2 = 10/kg lactated Ringers solution) 6 animals

Table 1. **Experimental Groups** This table gives the number of animals in each experimental group, and details the different treatment regimens that groups received.

animal #	#1	#2	#3	#4
*C.O.- baseline	161	122	204	174
C.O.- 2 hours after E.coli	123	131	213	150
C.O.- after **SFH (2.5 hours after E.coli)	70	55	161	114
C.O.- 5 hours after E.coli	dead	25	dead	dead

* C.O.= Cardiac output
** SFH = Stroma-free hemoglobin

Table 2. **Cardiac Output (CO) for Stroma-free hemoglobin group** This table shows no reproducible or predictive trends in the analysis of 4 animals' cardiac output after induction of sepsis, followed by treatment with 10 ml/kg stroma-free hemoglobin intravenously.

animal #	#1	#2	#3	#4	#5
*C.O.- baseline	141	91	106	132	130
C.O.- 2 hours after E.coli	121	146	136	118	92
C.O.- after ** LR (2.5 hours after E.coli)	110	119	186	147	103
C.O.- 5 hours after E.coli	dead	dead	163	dead	77

* C.O.= Cardiac output
** L.R.= Lactated Ringer's

Table 3. Cardiac Output (CO) for lactated Ringer group This table shows no reproducible or predictive trends in the analysis of 4 animals' cardiac output after induction of sepsis, followed by treatment with 10 ml/kg Ringer's lactate solution intravenously.

tissue type	Brain	Heart	Portal	Skin	Muscle
% of C.O.- baseline*	0.81	3.26	5.38	0.12	0.10
% of C.O.- 2 hours after E.coli	1.13	4.79	1.13	0.07	0.16
% of C.O.- after ** LR (2.5 hours after E.coli)	1.11	4.55	1.04	0.06	0.19
% of C.O.- 5 hours after E.coli	1.16	4.05	1.29	0.08	0.04

* C.O.= Cardiac output
** L.R.= Lactated Ringer's

Table 4. Regional blood flow for lactated Ringer's group - This table shows no reproducible or predictive trends in the analysis of 4 animals' tissue blood flow after induction of sepsis, followed by treatment with 10 ml/kg Ringer's lactate solution intravenously.

tissue type	Brain	Heart	Portal	Skin	Muscle
% of C.O.- baseline**	0.8	2.5	1.13	0.11	0.06
% of C.O.- 2 hours after E.coli	1.0	2.8	1.04	0.07	0.06
% of C.O.- after * SFH (2.5 hours after E.coli)	0.87	2.84	0.76	0.08	0.06
% of C.O.- 5 hours after E.coli	0.74	2.83	2.12	0.02	0.03

* SFH = Stroma-Free Hemoglobin

** C.O.= Cardiac output
** L.R.= Lactated Ringer's

Table 5. Regional blood flow for Stroma-free hemoglobin group - This table shows no reproducible or predictive trends in the analysis of 4 animals' tissue blood flow after induction of sepsis, followed by treatment with 10 ml/kg stroma-free hemoglobin solution intravenously.